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(54) Title: ASSOCIATION OF THE SEROTONIN TRANSPORT (HTT) GENE WITH CARDIOVASCULAR DISEASE AND LONGEVITY

(57) Abstract

Disclosed are methods for screening subjects to determine their risk for developing cardiovascular disease, screening methods to determine potential longevity, therapeutic methods and compositions for treating patients at risk for developing cardiovascular disease, and screening methods for identifying materials useful in the therapeutic methods.

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TITLE OF THE INVENTION

ASSOCIATION OF THE SEROTONIN TRANSPORT (HTT) GENE WITH CARDIOVASCULAR DISEASE AND LONGEVITY

CROSS-REFERENCES TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/110,150 filed November 25, 1998, and U.S. Provisional Application No. 60/075,613 filed February 20, 1998.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

Not applicable.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to the association between the serotonin transporter (HTT) gene and serum cholesterol levels, heart disease and longevity.

2. Description of the Related Art

The full citations of the publications referred to herein are found at the end of the specification. The contents of the references are incorporated herein by reference.

Low cholesterol levels have been reported to be associated with the development of depression and violent death by suicide, especially in the elderly (1-5). This occurs both in subjects in the general population and subjects whose cholesterol levels have been lowered by medication. Several possible mechanisms for these phenomena have been suggested including a decrease in serotonin levels (3, 6) or a decrease in the number of

membrane serotonin receptors or transporters due to the effect of low cholesterol on membrane fluidity (6). A link between serotonin levels and cholesterol was supported by studies in monkeys showing that those with cholesterol levels altered by diet showed a positive correlation between plasma cholesterol level and central serotonergic activity (7, 8). By contrast, a study by Fernstron, *et al.* (9) found no significant differences in tryptophan, serotonin or 5-HIAA concentrations in several brain regions in gerbils with a wide range of diet induced variations in cholesterol level.

Steegmans, *et al.* (10) reported a significant decrease in plasma serotonin, but not platelet serotonin, in 100 men in the general population with a demonstrated long term (3 years) cholesterol level below the fifth percentile compared to 100 control men with cholesterol levels in the 35th to 75th percentile. Smith and Betteridge (11) observed a significant negative correlation between platelet serotonin and cholesterol levels in subjects with hypercholesterolemia and controls (r for both combined = $-.48$, $p \leq 0.005$). In the hypercholesterolemic subjects there was a significant positive correlation with high-density lipoprotein (HDL) ($r = .79$, $p = .001$). They concluded there was a significant relationship between circulating cholesterol and platelet serotonin and that a serotonin uptake (transporter) mechanism was involved. Others have suggested the apparent association between low cholesterol and depression could be due to the fact that both were related to a third confounding factor, such as general poor health (12).

The observations that low cholesterol levels induced by medication or diet can be associated with depression suggest that environmental factors are involved and that

the low cholesterol was the primary event while the altered serotonin levels were secondary. However, the observation that low cholesterol levels in individuals in the general population can be associated with low serotonin levels and depression is compatible with the possibility that in some cases genetic factors could be responsible for either the low cholesterol, the depression, or both. The fact that elderly subjects are often involved suggests that some variables may be related to age. There is also a well documented association between depression and cardiovascular disease in general (13).

BRIEF SUMMARY OF THE INVENTION

In one aspect, the present invention relates to a method for screening a subject to determine whether such subject is at increased risk for developing cardiovascular disease, said method comprising determining the subject's genotype with respect to the serotonin transport (HTT) gene, wherein an LS heterozygote for the HTTLPR insertion/deletion polymorphism at the promoter region of the HTT gene has an increased risk for developing said disease.

In another aspect, the present invention relates to a method for screening a subject to determine whether such subject is a candidate for a therapy using a drug which prevents or treats a cardiovascular disease associated with excessive production of the serotonin transport protein, said method comprising determining the subject's genotype with respect to the serotonin transport (HTT) gene, wherein an LS heterozygote for the HTTLPR insertion/deletion polymorphism at the promoter region of the HTT gene is a candidate for such therapy.

In another aspect, the present invention provides a method for screening a subject to determine the potential longevity of such subject, said method comprising determining the subject's genotype with respect to the serotonin transport (HTT) gene, wherein an SS homozygote for the HTTLPR insertion/deletion polymorphism at the promoter region of the HTT gene has a greater probability of survival past eighty years of age.

In another aspect, the present invention relates to a method for treating a patient at increased risk for developing cardiovascular disease due to the patient being LS heterozygous for the HTTLPR insertion/deletion polymorphism at the promoter region of the serotonin transport (HTT) gene, said method comprising administering to said patient an effective amount of a material which diminishes the effect of the serotonin transporter protein.

In another aspect, the present invention relates to a method for identifying materials that can be used in the treatment of a patient at increased risk for developing cardiovascular disease due to the patient being LS heterozygous for the HTTLPR insertion/deletion polymorphism at the promoter region of the serotonin transport (HTT) gene, said method comprising determining whether the material is capable of diminishing the effect of the serotonin transporter protein.

In another aspect, the present invention relates to a pharmaceutical composition which comprises

a) an effective amount of a material which is capable of diminishing the effect of the serotonin transporter protein in a patient being LS heterozygous for the HTTLPR insertion/deletion polymorphism at the promoter region of the serotonin transport (HTT) gene; and

b) a pharmaceutically acceptable carrier.

In another aspect, the present invention relates to a kit suitable for screening a subject to determine whether such subject is at increased risk for developing cardiovascular disease, said kit comprising

a) means for determining the subject's genotype with respect to the insertion/deletion polymorphism at the promoter region of the serotonin transport gene;

b) suitable packaging material; and optionally

c) instructional material for use of said kit.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the postulated relationships between the HTT genotypes, cholesterol, depression, and cardiovascular disease.

Figure 2 shows a partial sequence of the regulatory region of the serotonin transporter gene. [SEQ.ID.NO:1]

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the observation that subjects having the LS heterozygote for the insertion/deletion polymorphism in the promoter region of the serotonin transporter (HTT) gene have an increased risk of developing cardiovascular disease, such as elevated cholesterol, angina and heart attacks. Additionally, subjects having the SS homozygote have a greater probability of survival past eighty years of age.

The present invention entails the determination of the subject's genotype with respect to the insertion/deletion polymorphism of the HTT gene described above. Such can be determined, for example, by analysis of the subject's DNA, RNA, or protein, with DNA analysis being particularly preferred. Suitable analysis

techniques are well known to those in the art, and include amplification genotyping (amplification of the desired region by suitable methods, such as PCR, followed by electrophoresis), *in situ* hybridization techniques, direct DNA sequencing, *etc.*

The L and S alleles of the human serotonin gene which are detected in practice of the present invention are described in reference 14, the contents of which are incorporated herein by reference. The relevant portion of the sequence is shown in Figure 2 herein. The L allele is shown, and the portion of that allele which is deleted in the S allele is indicated under "Deletion". The complete protein and cDNA sequences are reported (*J. Neural Transm.* 91, 67-73 (1993), incorporated herein by reference) as follows:

/translation="METTPLNSQKQLSACEDGEDCQENGVLQKVVPTPGDKVESGQI
S

NGYSAVPSPGAGDDTRHSIPATTTTLVAELHQGERETWGKKVDFLLSVIGYAVDLGN
V

WRFPYICYQNGGGAFLLPYTIMAIFGGIPLFYMELALGQYHRNGCISIWRKICPIFK
G

IGYAICIIAFYIASYYNTIMAWALYYLISSFTDQLPWTSCKNSWNTGNCTNYFSEDN
I

TWTLHSTSPAEEFYTRHVLQIHRSKGLQDLGGISWQLALCIMLIFTVIYFSIWKGVK
T

SGKVVWVTATFPYIILSVLLVRGATLPGAWRGVLFYLKPNWQKLLETGVWIDAAAQI
F

FSLGPGFGVLLAFASYNKFNNNCYQDALVTSVVNCMTSFVSGFVIFTVLGYMAEMRNE

DVSEVAKDAGPSLLFITYAEAIANMPASTFFAIIFFLMLITLGLDSTFAGLEGVITA
V

LDEFPHVWAKRRERFVLAVVITCFFGSLVTLTFGGAYVVKLLEEYATGPAVLTVALI
E

AVAVSWFYGITQFCRDVKEMLGFSPGWFWRICWVAISPLFLLFIICSFLMSPPQLRL
F

QYNYPYWSIILGYCIGTSSFICIPTYIAYRLIITPGTFKERIIKSITPETPTEIPCG
DIRLNAV"

BASE COUNT 459 a 574 c 541 g 525 t

ORIGIN

1 GCGTGCAACC CGACGATAGA GAGCTCGGAG GTGATCCACA AATCCAAGCA CCCAGAGATC

61 CATTGGGATC CTTGGCAGAT GGACATCAGT GTCATTTACT AACCAGCAGG ATGGAGACGA

121 CGCCCTTGAA TTCTCAGAAG CAGCTATCAG CGTGTGAAGA TGGAGAAGAT TGTCAGGAAA

181 ACGGAGTTCT ACAGAAGGTT GTTCCCACCC CAGGGGACAA AGTGGAGTCC GGGCAAATAT

241 CCAATGGGTA CTCAGCAGTT CCAAGTCCTG GTGCGGGAGA TGACACACGG CACTCTATCC

301 CAGCGACCAC CACCACCCTA GTGGCTGAGC TTCATCAAGG GGAACGGGAG ACCTGGGGCA

361 AGAAGGTGGA TTTCCTTCTC TCAGTGATTG GCTATGCTGT GGACCTGGGC AATGTCTGGC

421 GCTTCCCCTA CATATGTTAC CAGAATGGAG GGGGGGCATT CCTCCTCCCC TACACCATCA

481 TGGCCATTTT TGGGGGAATC CCGCTCTTTT ACATGGAGCT CGCACTGGGA CAGTACCACC

541 GAAATGGATG CATTTCAATA TGGAGGAAAA TCTGCCCGAT TTTCAAAGGG ATTGGTTATG

601 CCATCTGCAT CATTGCCTTT TACATTGCTT CCTACTACAA CACCATCATG GCCTGGGCGC

661 TATACTACCT CATCTCCTCC TTCACGGACC AGCTGCCCTG GACCAGCTGC AAGAACTCCT

721 GGAACACTGG CAACTGCACC AATTACTTCT CCGAGGACAA CATCACCTGG ACCCTCCATT

781 CCACGTCCCC TGCTGAAGAA TTTTACACGC GCCACGTCCT GCAGATCCAC CGGTCTAAGG

841 GGCTCCAGGA CCTGGGGGGC ATCAGCTGGC AGCTGGCCCT CTGCATCATG CTGATCTTCA

901 CTGTTATCTA CTTCAGCATC TGGAAAGGCG TCAAGACCTC TGGCAAGGTG GTGTGGGTGA

961 CAGCCACCTT CCCTTATATC ATCCTTTCTG TCCTGCTGGT GAGGGGTGCC ACCCTCCCTG

1021 GAGCCTGGAG GGGTGTTCTC TTCTACTTGA AACCCAATTG GCAGAAACTC CTGGAGACAG

1081 GGGTGTGGAT AGATGCAGCC GCTCAGATCT TCTTCTCTCT TGGTCCGGGC TTTGGGGTCC

1141 TGCTGGCTTT TGCTAGCTAC AACAAGTTCA ACAACAACTG CTACCAAGAT GCCCTGGTGA

1201 CCAGCGTGGT GAACTGCATG ACGAGCTTCG TTTCGGGATT TGTCATCTTC ACAGTGCTCG

1261 GTTACATGGC TGAGATGAGG AATGAAGATG TGTCTGAGGT GGCCAAAGAC GCAGGTCCCA

1321 GCCTCCTCTT CATCACGTAT GCAGAAGCGA TAGCCAACAT GCCAGCGTCC ACTTTCTTTG

1381 CCATCATCTT CTTTCTGATG TTAATCACGC TGGGCTTGGA CAGCACGTTT GCAGGCTTGG

1441 AGGGGGTGAT CACGGCTGTG CTGGATGAGT TCCCACACGT CTGGGCCAAG CGCCGGGAGC

1501 GGTTCGTGCT CGCCGTGGTC ATCACCTGCT TCTTTGGATC CCTGGTCACC CTGACTTTTG

1561 GAGGGGCCTA CGTGGTGAAG CTGCTGGAGG AGTATGCCAC GGGGCCCGCA GTGCTCACTG

1621 TCGCGCTGAT CGAAGCAGTC GCTGTGTCTT GGTTCTATGG CATCACTCAG TTCTGCAGGG

1681 ACGTGAAGGA AATGCTCGGC TTCAGCCCGG GGTGGTTCTG GAGGATCTGC TGGGTGGCCA

1741 TCAGCCCTCT GTTTCTCCTG TTCATCATTT GCAGTTTTCT GATGAGCCCG CCACAACTAC

1801 GACTTTTCCA ATATAATTAT CCTTACTGGA GTATCATCTT GGGTTACTGC ATAGGAACCT

1861 CATCTTTCAT TTGCATCCCC ACATATATAG CTTATCGGTT GATCATCACT CCAGGGACAT

1921 TTAAAGAGCG TATTATTAAA AGTATTACCC CGGAGACACC AACAGAAATT CCTTGTGGGG

1981 ACATCCGCTT GAATGCTGTG TAACACACTC ACCGAGAGGA AAAAGGCTTC TCCACAACCT

2041 CCTCCTCCAG TTCTGAGGAG GCACGCCTGC CTTCTCCCCT CCGAGTGAAT GAGTTTGCC

A further aspect of the present invention is the treatment of LS heterozygote patients at increased risk for developing cardiovascular disease to prevent the development or progression of such disease. The patient is administered an effective amount of a material which diminishes or eliminates the adverse effects of the serotonin transport protein produced by the patient. The material may act in a number of ways which would be apparent to one of ordinary skill. For example, it may act to decrease the production of the protein, such as by affecting the DNA or RNA responsible for protein production, or by affecting regulatory elements. One way to accomplish diminished protein production is by introduction via gene therapy or gene repair techniques of a gene or gene segment which converts an L allele into

either the S allele as described herein, or an allele having the same function as the S allele. See, for example, the techniques described in U.S. Patent No. 5,776,744, the contents of which are incorporated herein by reference. The material may also act by directly or indirectly affecting the produced protein to diminish the protein's activity or effect.

It will be apparent that the information regarding a subject's genotype with respect to the HTT gene may also be used to determine whether the subject is a candidate for a therapy using a drug which prevents or treats cardiovascular disease caused by excessive production of the serotonin transport protein.

For therapeutic treatment, the materials of the present invention may be formulated into a pharmaceutical composition, which may include, in addition to an effective amount of the active ingredient, pharmaceutically acceptable carriers, diluents, buffers, preservatives, surface active agents, and the like. Compositions may also include one or more other active ingredients if necessary or desirable.

The pharmaceutical compositions of the present invention may be administered in a number of ways as will be apparent to one of ordinary skill in the art. Administration may be done topically, orally, by inhalation, or parenterally, for example.

Topical formulations may include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Oral formulations include powders, granules, suspensions or solution in water or non-aqueous media, capsules or tablets, for example. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders may be used as needed.

Parenteral formulations may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives.

The dose regimen of the compounds or compositions of the present invention will depend on a number of factors which may readily be determined, such as severity and responsiveness of the condition to be treated.

The present invention also provides a screening method for identifying materials that may be used in the treatment of a patient at increased risk for developing cardiovascular disease due to the patient being LS heterozygous for the HTTLPR insertion/deletion polymorphism at the promoter region of the serotonin transport (HTT) gene. In practice of such a method, a candidate material is screened in an assay which determines whether the material is capable of diminishing the effect of the serotonin transporter protein. Suitable assays would be readily apparent to one of ordinary skill, including animal models and *in vitro* assays. The assays may be designed to test, for example, the effect of the material on the production of the serotonin transport protein, or its effect on the activity of the protein.

The present invention also provides a kit suitable for screening a subject for any of the purposes described above (*i.e.*, to determine whether such subject is at increased risk for developing cardiovascular disease; to determine the potential longevity of such subject; or to determine whether such subject is a candidate for the drug therapy described above). The kit comprises means for determining the subject's genotype with respect to the insertion/deletion polymorphism at the promoter region of the serotonin transport gene. Preferably, such means comprise at least two primers capable of

hybridizing to a region flanking the HTTLPR insertion/deletion polymorphism at the promoter region of the serotonin transport gene. The primers preferably are suitable for use in an amplification reaction, such as PCR. The kit additionally contains suitable packaging material, and optionally contains instructional material for use of the kit, result interpretation, *etc.* The kit may also contain additional reactants suitable for use with the primers, such as appropriate concentrations of deoxynucleotide triphosphates, suitable buffers, polymerization enzymes, *etc.*

The following non-limiting example is illustrative of the processes of the present invention.

EXAMPLE 1

The present study incorporated the following aspects. We had a unique opportunity to examine the potential role of genetic factors in the regulation of cholesterol levels in a healthy but elderly population of men by utilizing the participants in the Golden Games. This is a group of men over 55 years of age, who each year compete in a series of athletic competitions. Cholesterol levels and a history of the presence or absence of heart disease, angina, and heart attack, were available on these subjects (the GG group). As a replication group we also had available a group of subjects in good general health from the Loma Linda University Center for Health Promotion (the CHP group). Cholesterol and triglyceride levels were available on these subjects. As an additional replication group we examined a third group of subjects from Loma Linda University Hospital on whom a history of heart attacks was available (the LLHosp group). Since the subjects in the first two groups were in reasonably good health this

minimized the potential role of poor health as a factor in the regulation of cholesterol levels. We examined the serotonin transporter gene (HTT, SLC6A4) since the re-uptake of serotonin plays a role in the regulation of both blood serotonin (re-uptake into platelets) and brain serotonin (re-uptake into presynaptic neurons). A well-characterized insertion (L)/deletion (S) polymorphism (HTTLPR) at the promoter of the HTT gene was utilized since it is known to be associated with variations in HTT gene expression (14). Genetic variants of the HTT gene have been reported to be associated with mood disorders in some (15-19) but not all (20-22) studies. Since elevated cholesterol levels have been reported to be associated with a decreased risk for cardiovascular disease in elderly individuals (23-24), we examined the association between the HTT gene and cholesterol levels in the Golden Games subjects in two age groups 42 to 70 and >70 years of age. (There were too few subjects >70 years of to allow testing in the CHP and LLHosp group.)

The *HTT* Gene. The promoter of the human 5-hydroxytryptamine (serotonin) transporter gene (HTT) is regulated by an interplay between positive and negative regulatory elements (25). A GC-rich repetitious sequence is located in the proximal 5 'regulatory region of the human HTT gene which silences transcriptional activity in nonserotonergic cells, and contains positive response elements (26). Heils, *et al.* (14) reported a common insertion/deletion polymorphism of this repetitive element. The deletion (short or S allele) was present in approximately one-third of the Caucasian population. Expression studies with a human choriocarcinoma cell line showed that the non-deletion or long or L allele was

associated with three times the rate of expression of the serotonin transporter compared to the S allele.

A further relevant aspect is the reported presence of molecular heterosis at the HTT gene (27, 28). Molecular heterosis refers to a situation in which the heterozygotes for a polymorphic gene marker show a greater or lesser phenotypic effect than either homozygote 2S. Little, *et al.* (27) examined levels of [^{125}I]ß-CIT (citalopram) binding (fmol/mg) to the serotonin transporter in the dorsal and median raphe nuclei and substantia nigra of human controls and subjects with chronic cocaine use. They correlated levels of binding with the genotypes of the SS, LS, and LL alleles of the HTT LPR polymorphism of the HTT gene. This showed that [^{125}I]ß-CIT binding was lower in the LS heterozygotes than either the SS or LL homozygotes in all three regions. A two-way ANOVA was significant for genotype and region and genotype main effect ($p < .001$). They also examined serotonin transporter mRNA levels in these three regions. The levels were highest for LL subjects and equally low for LS and SS subjects. Thus, the molecular heterosis effect was specific to an end function of the HTT gene in terms of [^{125}I]ß-CIT binding.

If the association between cholesterol levels and depression was due to a confounding third factor, the HTT gene would qualify as such a factor. The above three groups allowed us to determine if the HTTLPR polymorphism of the HTT gene predicted cholesterol levels, re-test this association for cholesterol and triglycerides, test if the HTT gene was associated with heart attacks, re-test this association, and to determine if the risk was different in the subjects 70 years of age or less versus those over age 70.

Methods

The Golden Games Group. Each year a group of veterans, all of whom are over 55 years of age, compete in an athletic competition called the Golden Games. It is held in a different city each year and the participants are amenable to medical studies including blood drawing. The year that the Golden Games were held in Southern California we obtained blood samples for DNA and cholesterol testing. Of the 100 subjects in the present study, 74 percent were non-Hispanic Caucasian, 18 percent were African-American, and 8 percent were Hispanic or other. They ranged in age from 25 to 91 years. The mean age of the 58 subjects in the ≤ 70 age group was 63.9 years (S.D. 4.1 years). The mean age of the 42 subjects in the > 70 age group was 74.8 (S.D. 3.1). Subjects were questioned about the presence of a history of angina, heart attacks or hypertension. In addition to coding for the presence or absence of each disorder, subjects were also coded for the number of different cardiovascular disease problems and this allowed the scoring of the "heart disease" variable.

The Center for Health Promotion Group (CHP). The subjects from the CHP study consisted of 102 non-Hispanic Caucasians from the Loma Linda University Center for Health Promotion. The age, sex, weight, height, and waist-hip ratio were determined on each subject. The subjects ranged in age from 42 to 70 years of age with a mean age of 55.4 years (S.D. 7.5 years). A fasting blood sample was obtained for determination of cholesterol and triglycerides. The recruitment particularly targeted staff members of the Loma Linda University, and Loma Linda University Medical Center.

The Loma Linda Smoking Group. The subjects from the LLHosp group consisted of a random sample of 83 non-Hispanic Caucasian male inpatients from the Jerry L. Pettis Memorial Veterans Administration Center, Loma Linda, CA, acquired from a range of hospital wards. They consisted of individuals 42 to 74 years of age with a mean age of 57.0 years (S.D. 8.87 years). There were too few subjects in the > 70 age group to analyze separately. Data on the presence or absence of heart attacks were available.

In both studies coded samples of blood were sent to the Department of Medical Genetics at the City of Hope National Medical Center where the genetic studies were performed blind to clinical data. Both studies were approved by the IRBs of both institutions.

Laboratory tests. Cholesterol and triglyceride levels were determined using the REP Ultra-30 HDL, VLDL/LDL Cholesterol system of Helena Laboratories (Beaumont, TX). Polymerase chain reaction genotyping of the HTTLPR polymorphism of the HTT gene was performed using conditions and similar primers to those reported by Heils, *et al.* (14). The forward primer had the following sequence:
GGCGTTGCCG CTCTGAATGC [SEQ ID NO:2].
The reverse primerhad the following sequence:
TGGTAGGGTG CAAGGAGAAT [SEQ ID NO:3].
PCR amplification was carried out in a final volume of 25 µl containing the sample DNA, 10 mM deoxyribonucleotides, 0.1 mM of each primer, Tris-HCl, KCl, $(NH_4)_2SO_4$, 15 mM $MgCl_2$, 5X Q' solution, and 1.25 U of Taq DNA polymerase. PCR amplification was carried out with an initial denaturation step of 95°C for 5 minutes, 40 cycles of 95°C for 30 seconds, 64°C for 1 minute, and 72°C for 1 minute,

and final reannealing step at 72°C for 5 minutes. PCR products were run on a 7% (40% 29:1) polyacrylamide gel and visualized with ethidium bromide.

Statistics. The mean cholesterol and triglyceride levels for the different HTT genotypes were compared by ANOVA. The significance levels were determined on the basis of the F-ratio. A post hoc Tukey test indicated those means that were significantly different at $\alpha = .05$. Regression analysis of the correlation between cholesterol and triglyceride levels and genotype was performed scoring the HTT gene as LL or SS = 0, and LS = 1. To examine the percent of the variance for heart attack, heart disease, and angina, their presence was scored as 1, and their absence as 0. This allowed the determination or r^2, or the percent of the variance that was attributable to the HTT gene. Chi square analysis was used to compare the number of subjects with a history of heart disease, angina or heart attack in the GG group, and heart attacks in the LLHosp group, versus the LS and LL or SS HTT genotype groups. All statistical analysis was by the SPSS statistical package (SPSS, Inc. Chicago, II).

Results

The results for the 100 Golden Games subjects are shown in Table 1. Since there was no significant difference in the frequencies of the LL, LS, and SS genotypes in the different racial groups, we first examined all races together. The mean cholesterol was 231.41 mg/dl for the LS heterozygotes, versus 197.00 mg/dl for the LL and 206.36 mg/dl for the SS groups ($p \leq .0056$). When tested for heterosis by comparing LS heterozygotes to LL+SS homozygotes, the mean cholesterol for the homozygotes was 200.65 mg/dl ($p \leq .0017$). When

restricted to Caucasians the LS heterozygotes again had the higher mean cholesterol levels but the results were not significant.

When the subjects in the 55 to 70 year old group were examined for all races the mean cholesterol for the LS heterozygotes was 240.00 mg/dl, compared to 190.76 mg/dl for the LL homozygotes, and 201.00 mg/dl for the SS homozygotes ($p \leq .0005$). When tested for heterosis by comparing LS heterozygotes to LL+SS homozygotes, the mean cholesterol for the homozygotes was 194.00 mg/dl ($p \leq .0001$). When restricted to Caucasians the results were still significant for all three genotypes ($p \leq .011$) and for heterozygotes versus homozygotes ($p \leq .0026$).

By comparison, when the subjects of all races over age 70 were compared the mean cholesterol for the three groups was similar ($p \leq .82$) with the level for the LS heterozygotes being only modestly greater (220.68 mg/dl) than for the homozygotes (210.38 mg/dl) ($p \leq .53$). When restricted to Caucasians, the mean cholesterol for the LS heterozygotes was actually lower (210.84 mg/dl) than for the homozygotes (213.57 mg/dl) ($p \leq .87$).

In an attempt to replicate these findings, we examined the CHP subjects. In addition to cholesterol levels these subjects also had trigyceride levels. Table 2 shows these results. To allow comparison to the Golden Games yet still have an adequate number of subjects, we examined individuals in the 42 to 70 year age group. There were too few subjects in the > 70 year group for statistical analysis.

The mean cholesterol levels were highest for the LS heterozygotes (218.60 mg/dl), lowest for the LL homozygotes (197.44 mg/dl) and also lower for the SS homozygotes (205.46 mg/dl). While the F-values for all three genotypes were of borderline significance ($p \leq$

.055) the Tukey test showed that the LS values were significantly higher than the LL values at $\alpha = .05$. The test for heterosis by comparison of heterozygotes versus homozygotes was significant ($p \leq .019$).

The mean triglyceride levels were also highest for the LS heterozygotes (158.58 mg/dl). The levels for both the LL and SS homozygotes were similar (114.20 mg/dl and 115.20 mg/dl) and lower than for the LS heterozygotes. The F-value for all three genotypes was significant ($p \leq .018$). The test for heterosis by comparison of heterozygotes versus homozygotes was significant ($p \leq .0046$). When sex was used as a covariant it, sex itself was not significant for either cholesterol or triglyceride levels. When BMI was used as a covariant the association between the LS subjects and elevated cholesterol remained significant for both groups ($p < .0001$ for the GG group, $p < .03$ for the CHP group).

For the Golden Games subjects who were 70 years of age or less, the HTT gene accounted for 23.3 percent of the variance of cholesterol levels, $p \leq .0001$; 10.2 percent of the variance for heart attack, $p \leq .016$; 9.9 percent of the variance for heart disease, $p \leq .018$; and 8.0 percent of the variance for angina, $p \leq .034$. For the CHP subjects, the HTT gene accounted for 5.3 percent of the variance of the cholesterol levels ($p \leq .019$), and 9.6 percent of the variance of the triglyceride levels ($p \leq .0066$).

To examine the hypothesis that the differences we observed by age group might be due to a selection process, we also examined the association between the presence of the HTT genotypes and history of heart disease, angina or heart attack for the two age ranges in the GG group. The results are shown in Table 3. For the GG group subjects less than 70 years of age, the

frequencies for those with the respective conditions in those with the LS versus the LL or SS genotype, were as follows: heart disease 68.4% versus 35.1%; angina 42.1% versus 16.2%, and heart attack 42.1% versus 13.5%. The increase in the frequency for those with the LS genotype was significant for all three diagnostic groups ($p \leq .016$ to $\leq .034$). By comparison, for those over 70 years of age there was no significant difference in the frequency for any of these heart problerns in those with the LS genotypes versus the LL or SS genotypes ($p \leq .31$ to 1.0).

In an attempt to replicate the association between the HTTalleles and heart attacks, we examined the LLHosp group (Table 3). Here 23.4% of the LS subjects had a history of a heart attack versus 5.6% of the LL or SS subjects ($p > .030$).

Discussion

Since cholesterol-lowering agents have been reported to be associated with increased violence, depression and suicide, the assumption has been that the changes in cholesterol levels are the primary effect and that changes in serotonin or other neurotransmitters, presumed to be the cause of the behavioral problems, are secondary. The present demonstration of a correlation between genetic variants of the HTT gene and serum cholesterol levels, heart disease, angina, and heart attacks, suggests that the low cholesterol and the increased depression might not be directly related but due to a third confounding factor, genetic variants of the HTT gene. While the mechanism for the association between the HTT gene and depression can be understood to be a result of the effect of the genetic variants on serotonin levels, the mechanism for the association between the HTT gene and cholesterol or triglyceride

levels is not intuitively obvious. The following are some of the possible explanations.

1. Serotonin has a direct effect on serum cholesterol levels. This would imply that blood or brain serotonin levels have a direct effect on the synthesis of degradation of cholesterol. We are unaware of such a mechanism.

2. Serotonin has an indirect effect on serum cholesterol levels. There are several possibilities.

a. Genetic variants of the HTT gene have been shown to be associated with mood. If LS heterozygosity was associated with depression, and depression was associated with life style changes that resulted in eating a high cholesterol diet, this could provide an indirect mechanism bv which the HTT gene could be associated with high cholesterol levels. The problem with this is that the association we observed between the HTTPRL polymorphism of the HTT gene is far more robust than any reports of an association between this polymorphism and depression.

b. A more likely possibility is that through the well-known effect of serotonin on appetite, genetic variants of the HTT gene could be associated with obesity, which in turn is highly correlated with cholesterol levels. However, the one study of the possible association between the HTTLPR polymorphism and obesity was negative (29). To determine if the HTTLPR polymorphism was associated with weight we examined the association with an age

normalized BMI in the CHP aroup. There was a significantlv higher BMI (26.0 ± 5.0) in those with the LL genotype compared to those with LS (23.1 ± 4.3) or SS (23.0 ± 5.5) genotypes ($p \leq 0.015$). Thus, while these results supported a role of the HTT gene in BMI, they did not support the hypothesis that the association of the LS genotype with elevated cholesterol levels was secondary to greater obesity in these individuals. Since abdominal obesity is most often associated with hypercholesterolemia and heart disease (30) we also examined the association between the HTTLPR polymorphism and the age normalized waist-hip ratios in the CHP group. This ratio was highest for those with the LS genotvpe (.89 ± .10) compared to those with the LL (.85 ± .09) or the SS (.86 ± .09). However, this was not significant ($p \leq .25$).

3. The indirect effect is through a third confounding covariant. The identity of this possible confounding covariant is unknown.

When all factors are considered, we believe the most likely explanation for the association between genetic variants of the HR gene and serum cholesterol levels is through the effect of serotonin on appetite and abdominal obesity. However, while a larger series of cases might show a significant correlation between the HR gene and abdominal obesity, as measured by waist:hip ratio, our results suggest it will not be as robust as the association with cholesterol and triglyceride levels. This suggests other factors associated with a more direct effect of the HTT gene on cholesterol levels and heart disease are involved. These potential interactions

between genotypes of the HTT gene, cholesterol levels, depression, and cardiovascular disease are summarized in Figure 1. The LS genotype may exert part of its effect on cholesterol levels and cardiovascular disease through an effect on appetite. There may also be an unknown direct effect on cholesterol levels and on abdominal obesity. Much of the association between the LS genotype and cardiovascular disease may be through the well known role of serotonin on vascular constriction, essential and pulmonary hypertension, platelet aggregation, thrombosis and atheromata formation (31-35). By contrast, the S allele and the SS genotype, may independently exert an effect on serum cholesterol levels and, through its regulation of synaptic serotonin levels, depression.

In addition to the association of the HTTLPR polymorphism with cholesterol and triglyceride levels, the other aspect of interest was the difference in the effect of the HR gene variants on subjects less than 70 years of age versus subjects greater than 70 years of age. This difference was only observed in the Golden Games subjects because they were the only group with a sufficient number of subjects over 70 to have statistical validity. The pattern for cholesterol levels, heart disease, angina, and heart attack were all similar. Thus, the effect was greater for LS heterozygotes for all of these variables only in the less than 70 age aroup. It disappeared or was negative (less effect in the LS heterozygotes) in the over 70 age group. While the number of subjects are still too small for definitive conclusions, we believe the most parsimonious explanation is that the LS heterozygotes who are at greatest risk tend to die at an earlier age. Thus, the LS heterozygotes with elevated cholesterol levels and

elevated risk for cardiovascular disease, are missing from the older age group.

A final aspect of this study is the question of whether it is relevant to the apparent association between low cholesterol levels and depression, or to the observation that an elevated cholesterol level becomes less of a risk factor for cardiovascular disease or premature death in older subjects. It would seem that since HTT gene variants are modestly associated with both affective disorders and serum cholesterol, the association between low cholesterol and depression could be due to the HTT gene as a confounding third factor. Based on the present results, the specific hypothesis would be that since LS heterozygosity is associated with elevated cholesterol levels, then LL or SS homozygosity, associated with lower cholesterol levels would be the genotypes associated with depression. The literature suggests this is the case. Those studies that have reported a positive association between the HTTLPR polymorphism and affective disorder have reported the association to be with the SS genotype, or S allele (36). Thus, in population based studies, individuals with the SS genotype would have lower cholesterol levels and greater levels of depression. However, since the S allele is associated with lower rates of synthesis of the 5-HT transporter, this should be associated with elevated levels of synaptic serotonin and less depression. This paradox has been commented on by Collier, *et al.* (18) and Routledge and Middlemiss (37). The latter authors suggested that the decreased expression of the H17 gene by the S allele results in a modest elevation of raphe serotonin levels, but this produces an enhanced negative feedback via somatodendritic 5-HT_{1A} receptors resulting in an overall decrease in terminal serotonin output.

The second issue is whether the present findings can explain the fact that an elevated cholesterol level is less of a risk factor for cardiovascular disease in older individuals. This decreased risk in older subjects seems to parallel our observation that the association of the LS genotype with elevated cholesterol levels, heart disease, angina, and heart attack also disappeared in the over 70 age group. Our hypothesis that the latter is due to the premature death of LS subjects, and could also explain why elevated cholesterol levels are no longer a risk factor in this age group, *i.e.* those LS subjects at greatest risk because of elevated levels of cholesterol and an elevated risk for heart disease associated with the HTT gene - die prematurely. Those who are left have elevated cholesterol levels due to non-genetic reasons or different genetic reasons, and in these subjects, perhaps because serotonin is not involved, an elevated cholesterol level *per se* does not have the same dire consequences. Further studies of the role of other serotonin genes in cardiovascular disease are in progress.

Table 1. Association between the Genotypes of the HTT Gene and Serum Cholesterol Levels in Golden Games Males in Two Age Groups

Genotype	N	Mean (mg/dl)	S.D.	F	p
Age All races (HTTLPR) (n = 100)					
LL	39	197.00	41.45		
LS	36	231.41*	48.30		
SS	25	206.36	49.10	5.48	≤.0056
LL+SS	64	200.65	48.30		
LS	36	231.41	48.30	10.36	≤.0017
Age Caucasians only (HTTLPR) (n = 72)					
LL	25	205.20	41.52		
LS	27	227.63*	40.82		
SS	20	208.05	53.55	1.89	≤.158
LL+SS	45	206.46	46.69		
LS	27	227.62	47.12	3.79	≤0.55
Age 55 to 70 All races (HTTLPR) (n = 58)					
LL	26	190.76	42.11		
LS	20	240.00#	44.05		
SS	12	201.00	28.41	8.72	≤.0005
LL+SS	38	194.00	38.22		
LS	20	240.00	44.05	17.07	≤.0001

Table 1 (continued)

Genotype	N	Mean	S.D.	F	p
Age 55 to 70 Caucasians only (HTTLPR) (n = 38)					
LL	16	199.37	39.95		
LS	14	243.21	44.93		
SS	8	202.00	28.71	5.11	≤.011
LL+SS	24	200.25	35.96		
LS	14	243.21	44.93	10.49	≤.0026
Age > 70 All races (HTTLPR) (n = 42)					
LL	13	209.46	38.67		
LS	16	220.68	52.58		
SS	13	211.31	63.45	.194	≤.82
LL+SS	26	210.38	51.51		
LS	16	220.68	52.58	.390	≤.53
Age > 70 Caucasians only (HTTLPR) (n = 34)					
LL	9	215.55	44.60		
LS	13	210.84	28.91		
SS	12	212.08	66.21	.025	≤.97
LL+SS	21	213.57	28.91		
LS	13	210.84	28.91	.025	≤.87

*Significantly different from LL by Tukey test at α = .05.

#Significantly different from LL and SS by Tukey test at α = .05.

Table 2. Association between the Genotypes of the HTT Gene and Serum Cholesterol and Triglyceride Levels in CHP Males and Females 42 to 70 years of age (n = 102)

Genotype	N	Mean (mg/dl)	S.D.	F	p
Cholesterol					
LL	34	197.44	32.98		
LS	55	218.60*	43.11		
SS	13	205.46	44.62	2.99	≤.055
LL+SS	47	199.65	36.23		
LS	55	218.60	42.78	5.65	≤.019
Triglycerides					
LL	34	114.20	71.76		
LS	55	158.58*	84.03		
SS	13	115.20	53.11	4.15	≤.018
LL+SS	47	114.55	66.56		
LS	55	158.58	84.03	8.39	≤.0046

*Significantly different from LL by Tukey test at α = .05.

Table 3. *HTT* Genotype and History of Angina, Heart Disease or Heart Attack (% yes or no, all 1 d.f.)

Condition |---*HTT Genotype*----|			Chi Square	p
GOLDEN GAMES				
A. Heart disease				
Age 55 to 70 All races (HTTLPR) (n = 56)				
	LS	**LL+SS**		
Yes	13 (68.4)	13 (35.1)		
No	6 (31.6)	24 (64.9)	5.59	≤.018
Age > 70 All races (HTTLPR) (n = 42)				
	LS	**LL+SS**		
Yes	8 (50.0)	13 (50.0)		
No	8 (50.0)	13 (50.0)	.000	≤1.00
B. Angina				
Age 55 to 70 All races (HTTLPR) (n = 56)				
	LS	**LL+SS**		
Yes	8 (42.1)	6 (16.2)		
No	11 (57.9)	31 (83.8)	4.48	≤.034
Age > 70 All races (HTTLPR) (n = 42)				
	LS	**LL+SS**		
Yes	5 (31.3)	8 (30.8)		
No	11 (68.8)	18 (69.2)	.001	≤.97

Table 3 (continued)

Condition |---*HTT Genotype*---|			Chi Square	p
C. Heart attack				
Age 55 to 70 All races (HTTLPR) (n - 56)				
	LS	**LL+SS**		
Yes	8 (42.1)	5 (13.5)		
No	11 (57.9)	32 (86.5)	5.75	≤.016
Age > 70 All races (HTTLPR) (n - 42)				
	LS	**LL+SS**		
Yes	6 (37.5)	6 (23.1)		
No	10 (62.5)	20 (76.9)	1.00	≤.31
LLHosp GROUP				
Heart attack (n = 83)				
	LS	**LL+SS**		
Yes	11 (23.4)	2 (5.6)		
No	36 (76.6)	34 (94.4)	4.91	≤.03

References

1. Golomb BA. Cholesterol and violence: is there a connection? Ann Int Med 1998; 128: 478-487.

2. Lindberg G, Rastam L, Guilberg B, Eklund GA. Low serum cholesterol concentration and short term mortality from injuries in men and women. BMJ 1992; 305: 277-279.

3. Brown SL, Dalive ME, Harris TB, Simonsick EM, Guralnik JM, Kohout FJ. Low cholesterol concentrations and severe depressive symptoms in elderly people. BMJ 1994; 308: 1328-1332.

4. Rozzini R, Bertozzi B, Barbisoni P, Trabucchi M. Low serum cholesterol and sertonin metabolism. Risk of depression is higher in elderly patients with lowest serum cholesterol values. BMJ 1966; 312: 1298-1299.

5. Ainiyet J, Rybakowski J. Low concentration level of total serum cholesterol as a risk factor for suicidal and aggressive behavior. Psychiatr Pol 1996; 30: 499-509.

6. Engelberg H. Low serum cholesterol and suicide. Lancet 1992; 339: 727-729.

7. Kaplan JR, Shively CA, Fontenot MB, et al. Demonstration of an association among dietary cholesterol, central serotonergic activity, and social behavior in monkeys. Psychosom Med 1994; 56: 479-484.

8. Muldoon M, Kaplan J, Manuck S, Mann J. Effects of a low-fat diet on brain serotonergic responsivity in cynomolgus monkeys. Biol Psychiatry 1992; 31: 739-742.

9. Fernstrom MH, Verrico CD, Ebaugh AL, Fernstrom JD. Diet-induced changes in serum cholesterol concentrations do not alter tryptophan hydroxylation rate of serotonin concentrations in gerbil brain. Life Sci 1996; 58: 1433-1444.

10. Steegmans PHA, Fekkes D, Hoes AW, Bak AAA, vender Does E, Grobbee DE. Low serum cholesterol concentration and serotonin metabolism in men. BMJ 1996; 312: 221.

11. Smith CC, Betteridge DJ. Reduced platelet serotonin content and release in familial hypercholesterolaemia. Atherosclerosis 1997; 130: 87-92.

12. Brown SL. Cholesterol concentrations and depression in elderly people. Ann Med 1994; 27: 141-142.

13. Musselman D, Eavan DL, Nemeroff CB. The relationship of depression to cardiovascular disease. Arch Gen Psychiatry 1998; 55: 580-592.

14. Heils A, Teuiel A, Petri S, et al. Allelic variation of human serotonin transporter gene expression. J Neurochem 1996; 66: 2621-2624.

15. Ogilvie AD, Battersby S, Bubb VJ, et al. Polymorphism in the serotonin transporter gene

associated with susceptability to major depression. Lancet 1996; 347: 731-733.

16. Rees M, Norton N, Jones I, et al. Association studies of bipolar disorder at the human serotonin transporter gene (hSERT; 5HTT). Mol Psychiatry 1997; 2: 398-402.

17. Furlong RA, Ho L, Walsh C, et al. Analysis and meta-analysis of two serotonin transporter gene polymorphisms in bipolar and unipolar affective disorders. Am J Med Gen (Neuropsych Genet) 1998; 81: 58-63.

18. Collier DA, Arranz MJ, Sham P, et al. The serotonin transporter is a potential susceptability factor for bipolar affective disorder. NeuroReport 1996; 7: 1675-1679.

19. Rosenthal NE, Mazanti CM, Barnett RL, et al. Role of serotonin transporter promoter repeat length polymorphism (5-HTTLPR) in seasonality and seasonal affective disorder. Mol Psychiatry 1998; 3: 175-177.

20. Kunugi H, Tatsumi M, Sakai T, Hatori M, Nanko S. Serotonin transporter gene polymorphism and affective disorder. Lancet 1996; 347: 1340.

21. Bellivier F, Laplanche J-L, Leboyer M, et al. Serotonin transporter gene and manic depressive illness: An association study. Biol Psychiatry 1997; 41: 750-752.

22. Greenberg BD, McMahon EF, Murphy DL. Serotonin transporter candidate gene studies in affective disorders and personality: promises and potential pitfalls. Mol Psychiatry 1998; 3: 186-189.

23. Curb JD, Schatz I, Grove J, et al. Association of short term mortality with plasma cholesterol in the elderly. Can J Cardiol 1997; Suppl 13: A0101.

24. Weverling-Rijnsburger AW, Blauw GJ, Lagaay AM, Knook DL, Meinders AE, Westendorp RG. Total cholesterol and risk of mortality in the oldest old. Lancet 1997; 350: 1119-1123.

25. Lesch KP, Balling U, Gross J, et al. Organization of the human serotonin transporter gene. J Neural Transm Gen Sect 1994; 95: 157-162.

26. Heils A, Teuiel A, Petri S, et al. Functional promoter and polyadenylation site mapping of the human serotonin (5-HT) transporter gene. J Neural Transm 1995; 102: 247-254.

27. Little KY, McLaughlin DP, Zhang L, et al. Cocaine, ethanol, and genotype effects on human midbrain serotonin transporter binding sites and mRNA levels. Am J Psychiatry 1998; 155: 207-213.

28. Comings DE, MacMurray JM. Molecular Heterosis: A Review. Am J Med Gen (Neuropsych Genet) 1998; (submitted)

29. Hinney A, Barth N, Ziegler A, et al. Serotonin transporter gene-linked polymorphic region: allele

distributions in relationship to body weight and in anorexia nervosa. Life Sci 1997; 61: PL 295-PL 303.

30. Larsson B, Svardsudd K, Welin L, Wilhelmsen L, Bjorntorp P, Tibblin G. Abdominal adipose tissue distribution, obesity, and risk of cardiovascular disease and death: 13 year follow up of participants in the study of men born in 1913. Br Med J (Clin Res Ed) 1984; 288: 1401-1404.

31. Yildiz O, Smith JR, Purdy RE. Serotonin and vasoconstrictor synergism. Life Sci 1998; 62: 1723-1732.

32. De Clerck F. The role of serotonin in thrombogenesis. Clin Physiol Biochem 1990; 8 Suppl 3: 40-49.

33. Vanhoutte P, Amery A, Birkenhager W, et al. Serotoninergic mechanisms in hypertension. Focus on the effects of ketanserin. Hypertension 1988; 11:111-133.

34. Amstein R, Fetkovska N, Luscher TF, Kiowski W, Buhler FR. Age and the platlet serotonin vasoconstrictor axis in essential hypertension. J Cardiovasc Pharmacol 1988; 11 Suppl 1: S35-S40.

35. Vanhoutte PM. Serotonin, hypertension and vascular disease. Neth J Med 1991; 38:35-42.

36. Collier DA, Stober G, Li T, et al. A novel functional polymorphism within the promoter of the serotonin transporter gene: possible role in

susceptibility to affective disorders. Molecular Psychiatry 1996; 1: 453-460.

37. Routledge C, Middlemiss DN. The 5-HT hypothesis of depression revisited. Molecular Psychiatry 1996; 1:437.

CLAIMS

1. A method for screening a subject to determine whether such subject is at increased risk for developing cardiovascular disease, said method comprising determining the subject's genotype with respect to the serotonin transport (HTT) gene, wherein an LS heterozygote for the HTTLPR insertion/deletion polymorphism at the promoter region of the HTT gene has an increased risk for developing said disease.

2. The method of claim 1, wherein the disease is selected from the group consisting of elevated cholesterol, angina and heart attack.

3. The method of claim 1, wherein said analysis is performed by sequencing.

4. The method of claim 1, wherein said analysis is performed by amplification of at least a portion of the promotor region.

5. The method of claim 1, wherein said analysis is performed by a hybridization reaction.

6. The method of claim 5, wherein the hybridization reaction is an *in situ* hybridization.

7. A method for screening a subject to determine the potential longevity of such subject, said method comprising determining the subject's genotype with respect to the serotonin transport (HTT) gene, wherein an SS homozygote for the HTTLPR insertion/deletion

polymorphism at the promoter region of the HTT gene has a greater probability of survival past eighty years of age.

8. The method of claim 7, wherein said analysis is performed by sequencing.

9. The method of claim 7, wherein said analysis is performed by amplification of at least a portion of the promotor region.

10. The method of claim 7, wherein said analysis is performed by a hybridization reaction.

11. The method of claim 10, wherein the hybridization reaction is an *in situ* hybridization.

12. A method for treating a patient at increased risk for developing cardiovascular disease due to the patient being LS heterozygous for the HTTLPR insertion/deletion polymorphism at the promoter region of the serotonin transport (HTT) gene, said method comprising administering to said patient an effective amount of a material which diminishes the effect of the serotonin transporter protein.

13. The method of claim 12, wherein the material causes a decrease in production of said protein.

14. The method of claim 12, wherein the material causes a decrease in the activity of said protein.

15. A method for identifying materials that can be used in the treatment of a patient at increased risk for developing cardiovascular disease due to the patient

being LS heterozygous for the HTTLPR insertion/deletion polymorphism at the promoter region of the serotonin transport (HTT) gene, said method comprising determining whether the material is capable of diminishing the effect of the serotonin transporter protein.

16. The method of claim 15, which comprises determining whether the material is capable of causing a decrease in production of said protein.

17. The method of claim 15, which comprises determining whether the material is capable of causing a decrease in the activity of said protein.

18. A pharmaceutical composition which comprises

a) an effective amount of a material which is capable of diminishing the effect of the serotonin transporter protein in a patient being LS heterozygous for the HTTLPR insertion/deletion polymorphism at the promoter region of the serotonin transport (HTT) gene; and

b) a pharmaceutically acceptable carrier.

19. The composition of claim 18, wherein the composition is capable of causing a decrease in production of said protein.

20. The composition of claim 18, wherein the composition is capable of causing a decrease in the activity of said protein.

21. A method for screening a subject to determine whether such subject is a candidate for a therapy using a drug which prevents or treats a cardiovascular disease

associated with excessive production of the serotonin transport protein, said method comprising determining the subject's genotype with respect to the serotonin transport (HTT) gene, wherein an LS heterozygote for the HTTLPR insertion/deletion polymorphism at the promoter region of the HTT gene is a candidate for such therapy.

22. A kit suitable for screening a subject to determine whether such subject is at increased risk for developing cardiovascular disease, said kit comprising

a) means for determining the subject's genotype with respect to the insertion/deletion polymorphism at the promoter region of the serotonin transport gene;

b) suitable packaging material; and optionally

c) instructional material for use of said kit.

23. The kit of claim 22, wherein component a) comprises at least two primers capable of hybridizing to a region flanking the HTTLPR insertion/deletion polymorphism at the promoter region of the serotonin transport gene.

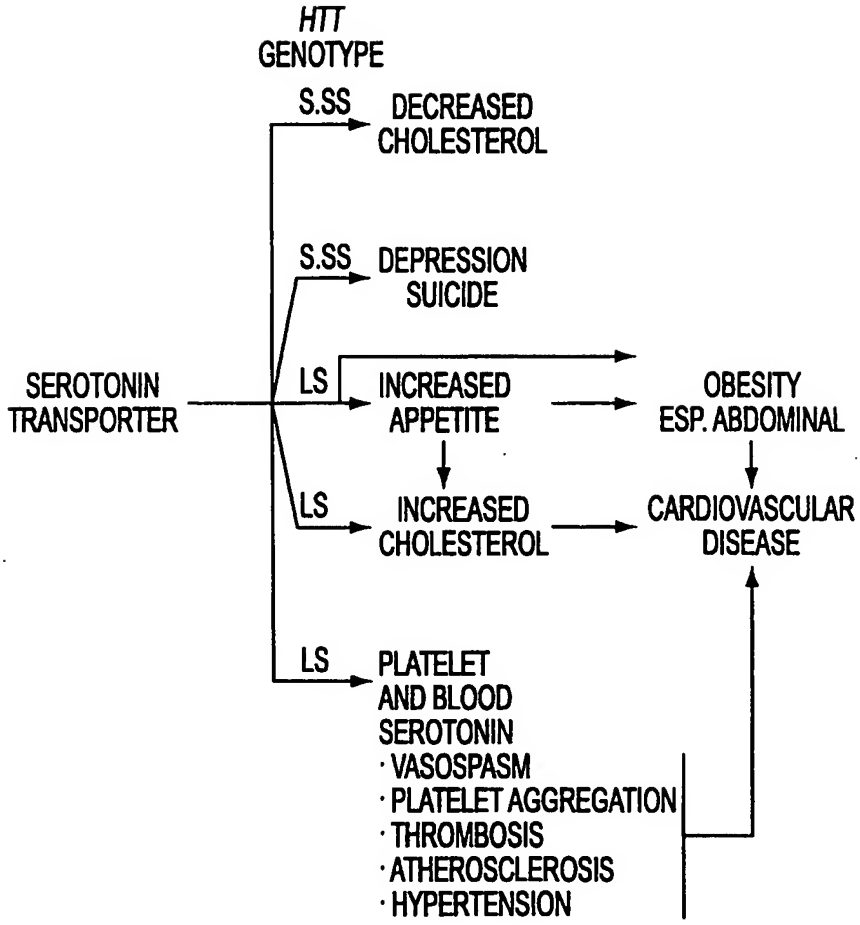
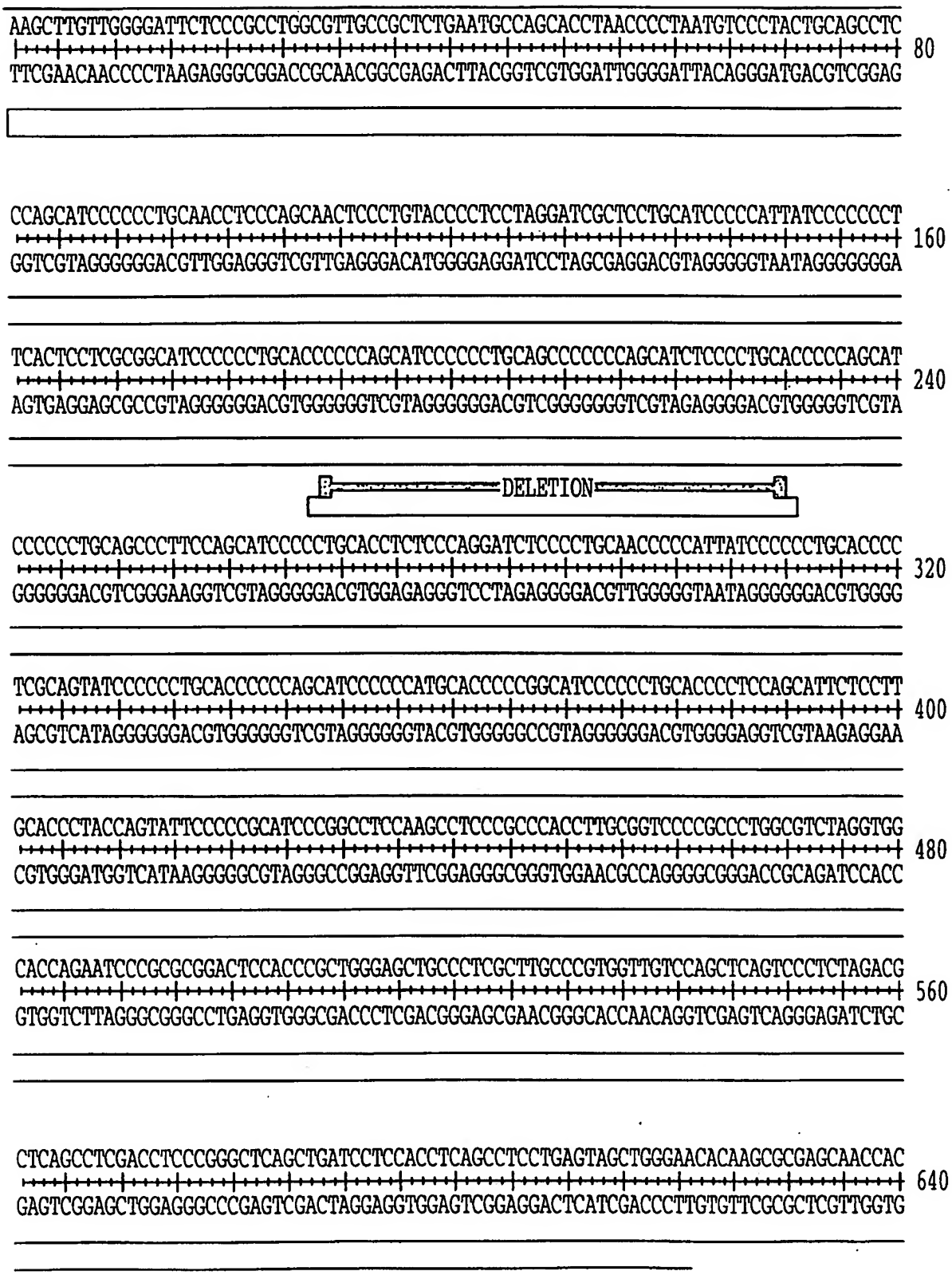

HTT
GENOTYPE
S.SS
DECREASED
CHOLESTEROL
S.SS
DEPRESSION
SUICIDE
SEROTONIN
TRANSPORTER
LS
INCREASED
APPETITE
OBESITY
ESP. ABDOMINAL
LS
INCREASED
CHOLESTEROL
CARDIOVASCULAR
DISEASE
LS
PLATELET
AND BLOOD
SEROTONIN
· VASOSPASM
· PLATELET AGGREGATION
· THROMBOSIS
· ATHEROSCLEROSIS
· HYPERTENSION

FIG. 1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160
TCACTCCTCGCGGCATCCCCCCTGCACCCCCCAGCATCCCCCCTGCAGCCCCCCCAGCATCTCCCCTGCACCCCCAGCAT
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240
DELETION
CCCCCCTGCAGCCCTTCCAGCATCCCCCTGCACCTCTCCCAGGATCTCCCCTGCAACCCCCATTATCCCCCCTGCACCCC
GGGGGGACGTCGGGAAGGTCGTAGGGGGACGTGGAGAGGGTCCTAGAGGGGACGTTGGGGGTAATAGGGGGGACGTGGGG
320
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400
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CGTGGGATGGTCATAAGGGGGCGTAGGGCCGGAGGTTCGGAGGGCGGGTGGAACGCCAGGGGCGGGACCGCAGATCCACC
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560
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GAGTCGGAGCTGGAGGGCCCGAGTCGACTAGGAGGTGGAGTCGGAGGACTCATCGACCCTTGTGTTCGCGCTCGTTGGTG
640

FIG. 2